**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of

**Heroux et al** Atty. Ref.: **2528-8**

Divisional of Serial No. **09/157,808** Group:

Filed: **October 15, 2001** Examiner:

For: **ASSAYS FOR MEASURING NUCLEIC ACID BINDING PROTEINS AND ENZYME ACTIVITIES**

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**October 15, 2001**

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

**PRELIMINARY AMENDMENT**

Preliminarily amend the above-identified application as follows.

**IN THE SPECIFICATION**

Amend the specification as follows:

Page 1, after the title, insert the following paragraph:

--This application is a divisional of Application No. 09/157,808, filed September 17, 1998, allowed, now U.S. Patent No. _____, the entire content of which is hereby incorporated by reference in this application.--

Page 9, the paragraph spanning lines 23-26, has been amended as follows:

--Figures 3(a), (b) and (c) show, schematically, three methods by which a substrate linked to a label and a binding reagent A can be contacted with a cleaving enzyme and a binding reagent B (on a solid phase) so as to form a first product linked to the label and a second product linked to the solid phase (by an A:B linkage).--

Page 10, the paragraphs spanning lines 1-8, have been amended as follows:

--Figures 4(a), (b) and (c) show, schematically, three methods by which a first substrate, linked to a binding reagent A, and a second substrate, linked to a label, can be contacted with a joining enzyme and a binding reagent B (on a solid phase) so as to form a product linked to both the label and the solid phase (by an A:B linkage).

Figures 5(a), (b), (c), (d), (e) and (f) illustrate six different embodiments of the invention for measuring the activity of cleaving enzymes.

Figures 6(a), (b), (c), (d) and (e) illustrate five different embodiments of the invention for measuring the activity of joining enzymes.--

Page 37, the paragraph spanning lines 18 and 19, has been amended as follows:

--5'-RuBpy-[AGTTGAGG]GGACTTT[CCCAGGC]-Biotin-3' (SEQ ID NO:1)
TCAACTCCCCTGAAAGGGTCCG-5' (SEQ ID NO:2)--.

Page 40, the paragraph spanning lines 10 and 11, has been amended as follows:

--**5'-Ruthenium**-GATCGAACTGACCGCCCGCGGCCCGT-**Biotin-3'** (SEQ ID NO:3)
CTAGCTTGACTGGCGGGCGCCGGGCA (SEQ ID NO:4)--.

Insert the attached Sequence Listing after the claims pages.

Insert the attached Abstract after the claims pages.

**IN THE CLAIMS**

Amend the claims as follows:

Cancel claims 1-44, without prejudice.

Add the following claims:

--45. (new) A method of assaying a sample for an activity that joins a first substrate with a second substrate to form a product comprising:

(a) forming a composition comprising said sample, said first substrate and said second substrate;

(b) incubating said composition under conditions wherein said activity can form said product;

(c) immobilizing a luminescent label on an electrode, wherein the luminescent label is linked to said product and said product is linked to said electrode, the immobilization being dependent on the formation of product;

(d) applying a voltage at said electrode so as to induce said immobilized luminescent label to emit luminescence; and

(e) measuring emitted luminescence so as to measure said activity.

46. (new) A method of assaying a sample for an activity that joins a first substrate with a second substrate to form a product comprising:

(a) forming a composition comprising said sample, said first substrate and said second substrate, said first substrate being linked to a luminescent label and said second substrate being linked to an electrode;

(b) incubating said composition under conditions wherein said activity can form said product, wherein said product is linked to said luminescent label and said electrode;

(c) applying a voltage at said electrode so as to induce said luminescent label in said product to emit luminescence; and

(d) measuring emitted luminescence so as to measure said activity.

47. (new) The method of claim 46, wherein said second substrate is linked to said electrode via an A:B linkage.

48. (new) The method of claim 46, wherein said electrode is further linked to one or more additional substrates, said second substrate and said one or more additional substrates forming a patterned array of substrates on the electrode, said patterned array of substrates comprising at least two regions that contain substrates that differ in structure.

49. (new) The method of claim 46, wherein said composition comprises one or more additional substrates, said second substrate and said one or more additional substrates being patterned on an array of independent electrodes, at least two electrodes containing substrates that differ in structure.

50. (new) A method of assaying a sample for an activity that joins a first substrate with a second substrate to form a product comprising:

(a) forming a composition comprising said sample, said first substrate and said second substrate, said first substrate being linked to a luminescent label and said second substrate being linked to a capture moiety;

(b) incubating said composition under conditions wherein said activity can form said product, wherein said product is linked to said luminescent label and said capture moiety;

(c) capturing said capture moiety on an electrode;

(d) applying a voltage at said electrode so as to induce said luminescent label in said product to emit luminescence; and

(e) measuring emitted luminescence so as to measure said activity.

51. (new) The method of claim 45, 46 or 50, wherein said activity results in the formation of a covalent bond.

52. (new) The method of claim 45, 46 or 50, wherein said first substrate or said second substrate comprises peptides.

53. (new) The method of claim 45, 46 or 50, wherein said first substrate or said second substrate comprises nucleic acids.

54. (new) The method of claim 45, 46 or 50, wherein said activity is the activity of a catalyst selected from the group consisting of nucleic acid polymerases, nucleic acid ligases, integrases, ribosomes, ubiquitin-protein ligases and trans-glutaminases.

55. (new) A method of assaying a sample for an activity that cleaves a substrate comprising:

(a) forming a composition comprising said sample and said substrate;

(b) incubating said composition under conditions wherein said activity can cleave said substrate;

(c) immobilizing a luminescent label on an electrode, wherein the luminescent label is linked to said substrate and said substrate is linked to said electrode, the immobilization being dependent on the presence of uncleaved substrate;

(d) applying a voltage at said electrode so as to induce said immobilized luminescent label to emit luminescence; and

(e) measuring emitted luminescence so as to measure said activity.

56. (new) A method of assaying a sample for an activity that cleaves a substrate comprising:

(a) forming a composition comprising said sample and said substrate, wherein said substrate is linked to a luminescent label and to an electrode;

(b) incubating said composition under conditions wherein said activity can cleave said substrate so as to cleave said luminescent label from said electrode;

(c) applying a voltage at said electrode so as to induce said luminescent label in said substrate to emit luminescence; and

(d) measuring emitted luminescence so as to measure said activity.

57. (new) The method of claim 56, wherein said substrate is linked to said electrode via an A:B linkage.

58. (new) The method of claim 56, wherein said electrode is further linked to one or more additional substrates, said substrate and said one or more additional substrates forming a patterned array of substrates on the electrode, said patterned array of substrates comprising at least two regions that contain substrates that differ in structure.

59. (new) The method of claim 56, wherein said composition comprises one or more additional substrates, said substrate and said one or more additional substrates being patterned on an array of independent electrodes, at least two electrodes containing substrates that differ in structure.

60. (new) A method of assaying a sample for an activity that cleaves a substrate comprising:

(a) forming a composition comprising said sample and said substrate, wherein said substrate is linked to a luminescent label and to a capture moiety;

(b) incubating said composition under conditions wherein said activity can cleave said substrate so as to cleave said luminescent label from said capture moiety;

(c) capturing said capture moiety on an electrode;

(d) applying a voltage at said electrode so as to induce said luminescent label linked to said substrate to emit luminescence; and

e) measuring emitted luminescence so as to measure said activity.

61. (new) The method of claim 55, 56 or 60, wherein said activity results in the cleavage of a covalent bond.

62. (new) The method of claim 55, 56 or 60, wherein said substrate comprises a peptide.

63. (new) The method of claim 55, 56 or 60, wherein said substrate comprises a nucleic acid.

64. (new) The method of claim 55, 56 or 60, wherein said activity is the activity of a catalyst selected from the group consisting of nucleases, proteases and glycosidases.

65. (new) The method of claim 45, 46, 50, 55, 56 or 60, wherein said electrode comprises elemental carbon.

66. (new) The method of claim 45, 46, 50, 55, 56 or 60, wherein said electrode comprises glassy carbon, carbon black, graphite, carbon fibers, carbon nanotubes or combinations thereof.

67. (new) The method of claim 45, 46, 50, 55, 56 or 60, wherein said electrode comprises conductive particles dispersed within or on a polymeric material.

68. (new) The method of claim 45, 46, 50, 55, 56 or 60, wherein said activity is an enzymatic activity.

69. (new) The method of claim 45, 46, 50, 55, 56 or 60, wherein said composition further comprises an inhibitor of said activity and the measurement of said activity is correlated to the amount or inhibitory ability of said inhibitor.

70. (new) An electrochemiluminescence assay for an activity that forms a covalent bond, wherein an electrochemiluminescence label is linked to an electrode via the covalent bond.

71. (new) An electrochemiluminescence assay for an activity that cleaves a covalent bond, wherein an electrochemiluminescence label is linked to an electrode via the covalent bond.

72. (new) The assay of claim 70 or 71, wherein said electrode is a carbon electrode.

73. (new) The assay of claim 70 or 71, wherein said electrode comprises glassy carbon, carbon black, graphite, carbon fibers, carbon nanotubes or combinations thereof.

74. (new) The assay of claim 70 or 71, wherein said electrode comprises conductive particles dispersed within or on a polymeric material.

75. (new) The assay of claim 70 or 71, wherein said activity is an enzymatic activity.

76. (new) The assay of claims 70 or 71 wherein said composition further comprises an inhibitor of said activity and the measurement of said activity is correlated to the amount or inhibitory ability of said inhibitor.

77. (new) A kit for measuring the activity of an enzyme that joins a first substrate and a second substrate, the kit comprising the enzyme, a solid phase, the first substrate, the second substrate and an electrochemiluminescence label, wherein said solid phase comprises an electrode that is linked to said first substrate and said electrochemiluminescent label is linked to said second substrate.

78. (new) A kit for measuring the activity of an enzyme that cleaves a substrate, the kit comprising the enzyme, a solid phase, the substrate and an electrochemiluminescence label, wherein said solid phase comprises an electrode, said substrate is linked to said electrode and said electrochemiluminescence label and said enzyme cleaves said substrate so as to form a first product linked to said electrode and a second product linked to said electrochemiluminescence label.

79. (new) The kit of claim 77 or 78, wherein said electrode is further linked to one or more additional substrates, so as to form a patterned array of substrates on the electrode, said patterned array of substrates comprising at least two regions that contain substrates that differ in structure.

80. (new) The kit of claim 77 or 78, wherein said solid phase comprises an array of independent electrodes and said kit further comprises one or more additional substrates, linked to said array of independent electrodes so that at least two electrodes contain substrates that differ in structure.--

## REMARKS

Claims 1-44 have been canceled, without prejudice.

Claims 45-80 have been added and are pending. Support for the pending claims may be found throughout the specification. No new matter has been added.

The specification has been amended above as provided in the parent application Serial No. 09/157,808. The attached formal drawings are the same as those submitted in the parent application. No new matter has been added. The attached paper copy of the Sequence Listing is the same as that filed in the parent application. The attached paper copy of the Sequence Listing is the same as the computer-readable copy of the Sequence Listing filed October 1, 1999, in the parent application Serial No. 09/157,808. The Office is requested to use the computer-readable form of the Sequence Listing from the parent application Serial No. 09/157,808 for the present case. A separate Request regarding the computer readable copy of the Sequence Listing is attached.

An early and favorable Action on the merits in the above-identified application is requested.

Respectfully submitted,

**NIXON & VANDERHYE P.C.**

By: _____

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## MARKED UP SPECIFICATION

Page 9, the paragraph spanning lines 23-26, has been amended as follows:

[Figure 3 shows] Figures 3(a), (b) and (c) show, schematically, three methods by which a substrate linked to a label and a binding reagent A can be contacted with [an] a cleaving enzyme and a binding reagent B (on a solid phase) so as to form a first product linked to the label and a second product linked to the solid phase (by an A:B linkage).

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[Figure 4 shows] Figures 4(a), (b) and (c) show, schematically, three methods by which a first substrate, linked to a binding reagent A, and a second substrate, linked to a label, can be contacted with a joining enzyme and a binding reagent B (on a solid phase) so as to form a product linked to both the label and the solid phase (by an A:B linkage).

[Figure 5 illustrates] Figures 5(a), (b), (c), (d), (e) and (f) illustrate six different embodiments of the invention for measuring the activity of cleaving enzymes.

[Figure 6] Figures 6(a), (b), (c), (d) and (e) illustrate five different embodiments of the invention for measuring the activity of joining enzymes.

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